Decreased serum vitamin D levels in children with asthma are associated with increased corticosteroid use

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Background: There is little knowledge about clinical variables associated with vitamin D (VitD) insufficiency in asthmatic children.

Objective: We sought to investigate disease variables associated with VitD insufficiency in patients with childhood asthma and interaction of VitD with corticosteroid-mediated anti-inflammatory responses.

Methods: We analyzed 25-hydroxyvitamin D serum levels in 100 asthmatic children to investigate relationships between 25-hydroxyvitamin D levels and patients' characteristics. We determined VitD's effects on dexamethasone (DEX) induction of mitogen-activated protein kinase phosphatase 1 and IL-10 in PBMCs.

Results: The median 25-hydroxyvitamin D serum level was 31 ng/mL. Forty-seven percent of subjects had VitD levels in the insufficient range (<30 ng/mL), whereas 17% were VitD deficient (<20 ng/mL). Log_{10} IgE ($P = .01$, $\rho = -0.25$) and the number of positive aeroallergen skin prick test responses ($P = .02$, $\rho = -0.23$) showed a significant inverse correlation with VitD levels, whereas FEV_1 percent predicted ($P = .004$, $\rho = 0.34$) and FEV_1/forced vital capacity ratio ($P = .01$, $\rho = 0.30$) showed a significant positive correlation with VitD levels. The use of inhaled steroids ($P = .0475$), use of oral steroids ($P = .02$), and total steroid dose ($P = .001$) all showed significant inverse correlations with VitD levels. The amount of mitogen-activated protein kinase phosphatase 1 and *IL10* mRNA induced by VitD plus DEX was significantly greater than that induced by DEX alone ($P < .01$). In an experimental model of steroid resistance in which DEX alone did not inhibit T-cell proliferation, addition of VitD to DEX resulted in significant dose-dependent suppression of cell proliferation.

Conclusions: Corticosteroid use and worsening airflow limitation are associated with lower VitD serum levels in asthmatic patients. VitD enhances glucocorticoid action in PBMCs from asthmatic patients and enhances the immunosuppressive function of DEX *in vitro*. (J Allergy Clin Immunol 2010;125:995-1000.)

Key words: *Vitamin D, children, asthma*

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Asthma is a chronic inflammatory disorder of the airways that causes an increase in airways hyperresponsiveness, leading to recurrent episodes of wheezing, breathlessness, and coughing that are associated with variable airflow obstruction.[1] According to data from the National Health Interview Survey, as of 2007, 8 million children between the ages of 5 and 17 years have been given diagnoses of asthma in their lifetimes.[2] In the United States asthma is the most common cause of childhood emergency department visits, hospitalizations, and missed school days.[3] Inhaled corticosteroids (ICSs) represent the preferred treatment for persistent asthma.[4] For patients with severe asthma who do not achieve adequate control with high-dose ICS therapy combined with a long-acting β-agonist, oral corticosteroids become a preferred treatment modality.[4] Although clearly effective in the management of asthma, corticosteroids come with a variety of potential side effects. Higher ICS doses increase the potential for systemic adverse effects that are seen with oral corticosteroids, including reduced bone density, increased fracture risk, and adrenal suppression.[5,6] Consequently, treatment options for asthma that reduce steroid doses can minimize the risk of these adverse effects.

One hypothesis for the increasing prevalence of asthma involves vitamin D (VitD). Some have argued that different factors associated with westernization have led to lower VitD levels, which in turn have resulted in higher rates of asthma.[7,8] However, others have argued that VitD has more of a deleterious effect on allergic pathogenesis.[9] Although multiple studies have examined maternal VitD status and subsequent wheezing in offspring,[8,10-11] there are limited data on VitD levels in children with asthma, as well as on what features of asthma are associated with VitD levels. A recent study on children with asthma from Costa Rica showed a significant inverse association between VitD levels and use of anti-inflammatory medication (either ICSs or leukotriene inhibitors) in the previous year, total IgE levels, and eosinophil counts.[12] These important findings require confirmation. To our knowledge, the prevalence of VitD insufficiency/deficiency is unknown for children with asthma living in higher northern latitudes. In addition, more information is needed regarding the specific clinical and therapeutic variables associated with lower VitD levels in patients with childhood asthma. The first aim of this article was to investigate the prevalence of

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Abbreviations used
DEX: Dexamethasone
FVC: forced vital capacity
ICS: inhaled corticosteroid
MKP-1: mitogen-activated protein kinase phosphatase 1
TSST: toxic shock syndrome toxin
VitD: vitamin D

VitD insufficiency in childhood asthma from a group of patients living in northern latitudes, as well as to further define what variables, including corticosteroid use and markers of allergy, are associated with VitD insufficiency in childhood asthma. The second part of this study was to determine whether VitD interacts directly with corticosteroid pathways that lead to downregulation of the inflammatory response. This was assessed by testing whether VitD enhances glucocorticoid induction of mitogen-activated protein kinase phosphatase 1 (MKP-1) and IL-10 in PBMCs by using real-time PCR and T-cell proliferation assays.

METHODS

Subjects

Children with asthma referred to National Jewish Health were identified through focused searches of laboratory data using codes for the 25-hydroxyvitamin D assay. Data were collected between April 1, 2008, and October 31, 2009. Patients' medical information was obtained by using the National Jewish Health electronic medical record and the National Jewish Research Database.[13] Patients between the ages of 0 and 18 years who had 25-hydroxyvitamin D serum levels (used interchangeably with VitD levels in this article) drawn were included if they had a physician's diagnosis of asthma. Patients were excluded if there was documentation that they were taking VitD supplements or if they had additional chronic pulmonary conditions (eg, cystic fibrosis or bronchiectasis). Laboratory studies to assess VitD's effects on corticosteroid action *in vitro* used 11 patients with mild-to-moderate asthma and 4 healthy control subjects. Approval was received from the National Jewish Health Institutional Review Board for both parts of the study.

Data collection

Serum 25-hydroxyvitamin D levels were analyzed with the VitD, 25-hydroxy chemiluminescent immunoassay performed at ARUP Laboratories (Salt Lake City, Utah). This assay is capable of measuring both the D_2 and D_3 derivates of 25-hydroxyvitamin D.[14] Values were reported in nanograms per milliliter. 25-Hydroxyvitamin D levels are the preferred marker of the body's VitD status because this form has a longer half-life (2-3 weeks) than 1,25-dihydroxyvitamin D (4 hours).[15] Skin testing was performed according to National Jewish guidelines by using histamine and saline controls. Positive reactions were recorded for wheal sizes greater than or equal to 3 mm in diameter larger than those elicited by the negative saline control. Seasonal aeroallergens tested were specific for the plants commonly found in the subject's home state. Total IgE level and eosinophil count measurements were performed by Advanced Diagnostics Laboratories (Denver, Colo) at National Jewish Health. Reflex titer assays were done to quantify IgE levels of greater than 5,000 kU/L. IgE levels underwent a $\log_{10}$ transformation for analysis. Eosinophil counts were determined by using the direct current electronic resistance method of particle counting and sizing. Latitude of the patient's home address was determined based on data from the United States Census Bureau Gazetteer Web site and the iTouchMap.com Web site. Exhaled nitric oxide levels were measured by using the NIOX system (Aerocrine, Solna, Sweden). Medication use and dosage was recorded. The total steroid dose was expressed as the average daily dose of ICSs plus oral corticosteroids taken over the 30 days before VitD assessment.

Laboratory studies were performed on purified PBMCs. Human PBMCs were isolated by means of Ficoll-Hypaque density gradient centrifugation. PBMCs were cultured in hormone-free medium containing 1,25-$(OH)_2D_3$ (10 nmol/L) for 24 hours, with dexamethasone (DEX; 10 or 100 nmol/L) added during the last 3 hours. Total RNA was extracted (Qiagen, Hilden, Germany), transcribed into cDNA, and analyzed by means of real-time PCR with the dual-labeled fluorogenic probe method on an ABI Prism 7300 Real Time PCR system (Applied Biosystems, Foster City, Calif). *MKP1*, *IL10*, and β-actin mRNA expression was determined. For proliferation studies, PBMCs were cultured in RPMI 1640 medium containing 10% FCS and stimulated with staphylococcal toxic shock syndrome toxin 1 (TSST-1; 100 ng/mL; Toxin Technology, Inc, Sarasota, Fla) for 72 hours to induce corticosteroid resistance, as described by us previously.[16] DEX (100 nmol/L) with or without 0.1, 1, 10, 50, or 100 nmol/L 1,25-$(OH)_2D_3$ was added to examine its effect on T-cell proliferation.

Statistical analysis

Population values for the variables examined are given in Table I. Univariate relationships between 25-hydroxyvitamin D levels and patients' demographic and therapeutic characteristics were determined by using the Spearman rank correlation coefficient when the variables were continuous and the Wilcoxon test with χ^2 approximation when they were categorical. These tests were chosen because of the nature of the retrospective convenience sample, the variability in sample size among variables, and the nonnormal distribution of variables. These univariate relationships are presented in Tables II and III. Variables were considered statistically significant at P values of less than .05 by using 2-sided tests. Statistical analysis was performed with JMP 8.0.1 software (SAS Institute, Inc, Cary, NC).

For the laboratory assessment of VitD's effects on corticosteroid action, data were expressed as means ± SEMs. The paired t test was used to compare functional responses of pre- and post-DEX–treated cells from the same donors (hence paired). The Wilcoxon matched pairs test was applied for samples that did not fit Gaussian distribution. A P value of less than .05 was considered statistically significant. All reported P values were based on 2-sided tests.

RESULTS

Subjects' characteristics

25-Hydroxyvitamin D levels and clinical features were analyzed in a total of 100 children with asthma aged 0 to 18 years (Table I). Racial data were available for 81 of the subjects. Seventy-nine percent of the participants were white, 9% were American Indian or Alaska Native (including Hispanic), 6% were African American, 3% were Asian, and 4% were of reported mixed race (data not shown).[17] The median latitude was 39.0°N. The median eosinophil counts and total IgE levels were 311 cells/mm^3 and 1,440 kU/L, respectively. The median for $\log_{10}$ IgE was 7.3. The median FEV_1 percent predicted was 93.8%, and the median FEV_1/forced vital capacity (FVC) ratio was 0.8. The date the 25-hydroxyvitamin D level was obtained was recorded to assess seasonal variations in the frequency of specimen collection. Subjects were broken down into a summer (March through October) or winter (November through February) designation. November through February was chosen for the winter season because very little VitD can be produced through sun exposure during these months in latitudes above 35°N.[18] Seventy-nine percent of the collections were done during months when cutaneous VitD synthesis was possible in northern latitudes (March through October).

Among all the asthmatic patients studied, the median serum 25-hydroxyvitamin D level was 31 ng/mL (Fig 1). Based on changes in parathyroid hormone levels and intestinal calcium transport that have been noted at VitD levels of less than 30 ng/mL,[18] values of less than 30 ng/mL were considered VitD insufficient. Forty-seven percent of the subjects studied had VitD levels in the insufficient range (<30 ng/mL), whereas 17% were VitD deficient

TABLE I. Patients' characteristics

Characteristic	Sample size	Data*
Age (y)	100	7 (4-10)
Sex	100	Male 64%
BMI percentile	98	60 (29.5-85.6)
Eosinophil count (cells/mm^3)	91	311 (146-576)
Log_{10} IgE	96	7.3 (6-9)
IgE (kU/L)	96	1,441 (386-7,678)
No. of positive aeroallergen skin test results	96	9 (4-16)
FVC (%)	69	99 (90.3-106.8)
FEV_1 (%)	69	93.8 (80.9-101.8)
FEV_1/FVC	69	0.8 (0.74-0.87)
Season	100	Summer 79%

BMI, Body mass index.
*Values are listed as a percentage of population or median (25th-75th interquartile range).

TABLE II. Univariate analysis of associations between serum VitD levels and continuous clinical variables*

Variable	Correlation*	*P* value
Age	−0.47	<.0001
BMI	−0.21	.04
Eosinophil count	−0.01	.91
Log_{10} IgE	−0.25	.01
No. of positive aeroallergen skin test results	−0.23	.02
FVC (%)	0.12	.33
FEV_1 (%)	0.34	.004
FEV_1/FVC	0.30	.01

BMI, Body mass index.
*Correlation was determined with the Spearman rank correlation coefficient.

(<20 ng/mL). Among the VitD-deficient patients, 88% had a 25-hydroxyvitamin D level of between 10 and 20 ng/mL.

Clinical associations

Tables II, III, and IV outline the results of the univariate analysis for variables associated with VitD levels. Tables II and III involve clinical variables. Table IV involves therapeutic (ie, medication) variables. A significant inverse correlation was noted for age and VitD level ($P < .0001$, $\rho = -0.47$). The median VitD value for female subjects was 33 ng/mL, and it was 30 ng/mL for male subjects. African Americans had a median VitD value of 24 ng/mL, whereas subjects who were not African American had a median value of 32 ng/mL. The differences in VitD levels with respect to sex and race were not significant, although only 6 subjects reported African-American race. Latitude and time of year (summer vs winter) were not significantly associated with VitD level (data not shown). Higher body mass index was associated with significantly lower VitD levels ($P = 0.04$, $\rho = -0.21$). Among markers for atopy, $\log_{10}$ IgE ($P = .01$, $\rho = -0.25$) and the number of positive environmental skin prick test responses ($P = .02$, $\rho = -0.23$) showed a significant inverse correlation with VitD levels, whereas eosinophil counts demonstrated no significant correlation. Sixty-nine of the participants had recorded spirometric results, with FEV_1 percent predicted ($P = .004$, $\rho = 0.34$) and FEV_1/FVC ratio ($P = .01$, $\rho = 0.30$) showing a significant positive correlation with VitD levels.

TABLE III. Univariate analysis of serum VitD levels and aeroallergen sensitivity*

Allergen	Positive skin test result (%)	VitD level†, median (IQR)	*P* value‡
Pollen (n = 93)	82	Positive: 29 (22-40) Negative: 31 (23-36)	.94
Dog (n = 95)	63	Positive: 29 (23-37) Negative: 35 (22-47)	.045
Cat (n = 95)	60	Positive: 29 (23-38) Negative: 31.5 (26-41)	.47
Mold (n = 95)	55	Positive: 28 (22-38) Negative: 34 (23-42)	.09
House dust mite (n = 92)	44	Positive: 27 (16-39) Negative: 31 (27-38)	.05
Alternaria species (n = 93)	39	Positive: 27.5 (20-34) Negative: 33 (25-40)	.06

IQR, Interquartile range.
*Positive aeroallergen sensitivity is defined as a positive skin test result to the allergen in question or any 1 positive skin test result to a specific allergen within the group in question.
†Vitamin D represents 25-hydroxyvitamin D. Categorical variables have comparison VitD medians expressed in nanograms per milliliter with 25th to 75th interquartile ranges in parentheses.
‡The Wilcoxon test was used for median value differences for categorical variables.

Separate univariate analyses were performed on individual aeroallergens and aeroallergen categories (Table III). Sensitivity to outdoor pollens was the most common skin test finding (82% of subjects) and was not associated with VitD levels. Mold allergens and *Alternaria* species in particular showed a trend toward lower VitD levels that did not achieve significance. However, sensitivity to the indoor aeroallergens dog ($P = .045$) and house dust mite ($P = .05$) were significantly associated with lower VitD levels.

Among the different therapeutic modalities assessed (Table IV), the use of inhaled steroids ($P = .0475$), use of oral steroids ($P = .02$), total steroid dose ($P = .001$), and use of long-acting β-agonists ($P = .0007$) all showed significant association with lower VitD levels. The use of leukotriene receptor antagonists, topical steroids, and multivitamins was not associated with VitD levels, although only 6 patients reported multivitamin use.

Laboratory assessment of VitD's effects on corticosteroid action

In unfractionated PBMCs, DEX induced *MKP1* expression (means ± SEMs) from 0.91 ± 0.17 ng MKP-1/ng β-actin for media to 8.51 ± 1.71 ng *MKP1*/ng β-actin for DEX-cultured cells, respectively (n = 11). There was a significant ($P < .001$) enhancement of DEX induction of *MKP1* by VitD (Fig 2, *A*). Steroid-sparing effects of VitD were noted because the amount of *MKP1* mRNA induced by VitD plus 10 nmol/L DEX combination was significantly greater than the amount induced by 100 nmol/L DEX alone ($P < .01$; Fig 2, *A*). Similar effects were observed for *IL10* (Fig 2, *B*).

To examine whether VitD can improve glucocorticoid action under corticosteroid-resistant conditions, PBMCs were stimulated with TSST-1 (100 ng/ml) for 72 hours to induce corticosteroid resistance, and DEX (100 nmol/L) or 1,25-$(OH)_2D_3$ (up to 100 nmol/L) was added alone or in combination to examine their effects on T-cell proliferation. TSST-1–stimulated T-lymphocyte proliferation in PBMCs was not inhibited by DEX alone or VitD

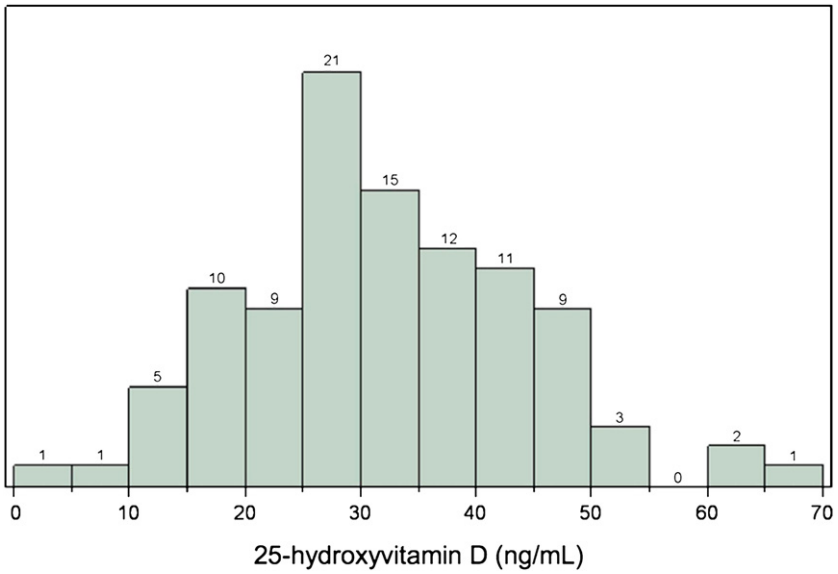

FIG 1. Distribution of VitD levels. VitD levels are expressed as serum 25-hydroxyvitamin D. The median value was 31 ng/mL. Forty-seven percent of asthmatic patients had insufficient levels of VitD (<30 ng/mL).

alone. However, addition of VitD to DEX resulted in significant dose-dependent suppression of TSST-induced cell proliferation, and up to 64% inhibition of cell proliferation was achieved ($P <$ .01; Fig 3).

DISCUSSION

In our population of pediatric patients with asthma, who were primarily from latitudes above 35°N, the prevalence of VitD insufficiency (<30 ng/mL) was 47%, with 17% of patients being VitD deficient (<20 ng/mL). These percentages were higher than in a recent study involving childhood asthma and VitD that was done in an equatorial population from Costa Rica, in which 28% had VitD insufficiency.[12] The majority of our patients (79%) had VitD levels collected during a time period in which cutaneous production of VitD is possible in higher northern latitudes (March through October). The differences between our study and the population from Costa Rica support the known association of increased risk of VitD deficiency in populations living at higher northern latitudes.[15] A study in infants and toddlers aged 8 to 24 months from an urban population in which the majority had darker skin pigmentation found that 40% of the participants were VitD insufficient (≤30 ng/mL).[19] In an adolescent urban population, again with a majority of subjects with darker skin pigmentation, 42% of the participants had VitD levels of less than 20 ng/mL.[20] Both of these studies were done on populations residing in northern latitudes. A recent article describing 25-hydroxyvitamin D levels in children using the National Health and Nutrition Examination Survey from 2001-2004 showed that 61% of children aged 1 to 21 years had insufficient levels of VitD.[21] Overall, the prevalence of VitD insufficiency in children with asthma from our study population is similar to that seen in general pediatric populations living at similar northern latitudes.

Our univariate analysis demonstrated several statistically significant variables. Age and body mass index both had significant inverse correlations with serum VitD levels. These findings are consistent with prior data.[18,21] In terms of lung function, FEV_1 percent predicted and FEV_1/FVC ratio were also significantly correlated with VitD level. In a study using an adult National Health and Nutrition Examination Survey sample of the general population, subjects whose VitD levels were in the highest quintile had significantly higher FEV_1 and FVC values.[22] Some elements of atopy, such as $\log_{10}$ IgE level and the number of positive aeroallergen skin test results, were inversely correlated with VitD levels in serum, whereas eosinophil counts were not.

TABLE IV. Univariate analysis of serum VitD levels and medication use

Medication used, sample size (n = 100)	VitD level,† median (IQR)	*P* value*
ICS (n = 60)	ICS: 29 (21-36) No ICS: 35 (26-42)	.0475
Oral CS (n = 14)	Oral CS: 25 (18-30) No oral CS: 32 (25-40)	.02
TCS (n = 69)	TCS: 31 (23-40) No TCS: 31 (24-38)	.98
LTRA (n = 42)	LTRA: 29 (25-37) No LTRA: 32 (23-41)	.50
LABA (n = 29)	LABA: 25 (19-31) No LABA: 34 (27-42)	.0007
MVI (n = 6)	MVI: 40 (27-53) No MVI: 31 (23-39)	.12
Total steroid dose (mg)‡ (n = 64)	$\rho = -0.32$§	.001

CS, Corticosteroid; *IQR*, interquartile range; *LABA*, long-acting β-agonist; *LTRA*, leukotriene receptor antagonist; *MVI*, multivitamin; *TCS*, topical corticosteroid.
*The Wilcoxon test was used for median value differences for categorical variables. Correlation was determined with the Spearman rank correlation coefficient for continuous variables.
†VitD represents 25-hydroxyvitamin D. Categorical variables have comparison VitD medians expressed in nanograms per milliliter with 25th to 75th interquartile ranges in parentheses.
‡Total steroid dose includes inhaled plus oral corticosteroids.
§Correlation was determined with the Spearman rank correlation coefficient for total steroid dose in the last row.

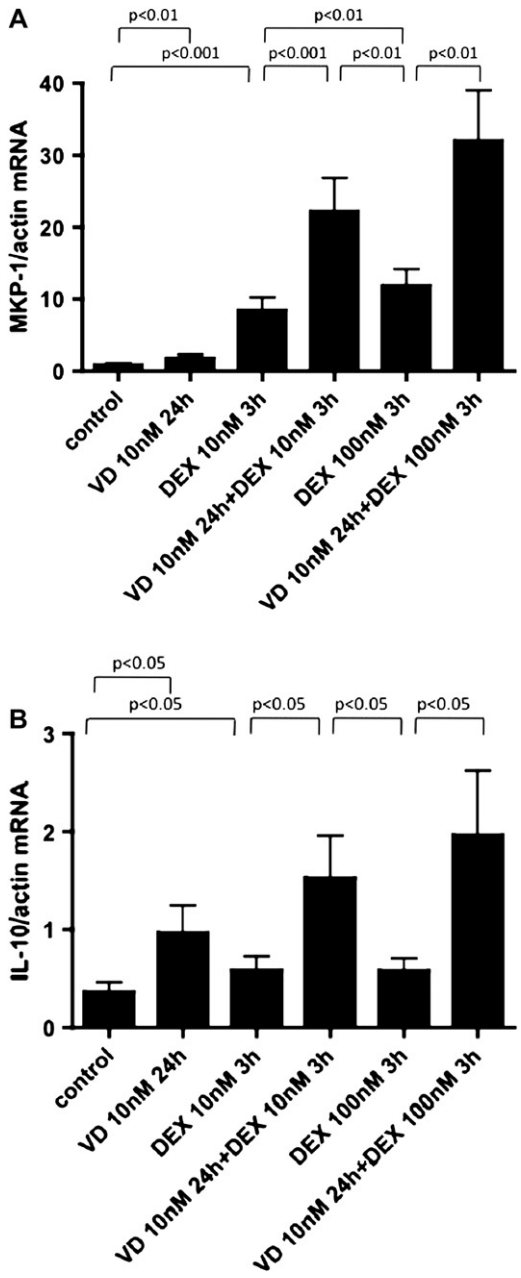

FIG 2. VitD *(VD)*–potentiated DEX-mediated transactivation in human PBMCs enhances DEX induction of *MKP1* **(A)** and *IL10* **(B)** mRNA. PBMCs from patients with asthma were cultured with 10 nmol/L VitD or media alone for 24 hours and supplemented with 10 or 100 nmol/L DEX for the last 3 hours of culture. *MKP1* mRNA levels were detected by means of real-time PCR and were normalized to actin mRNA. Values represent means ± SEMs (n = 11).

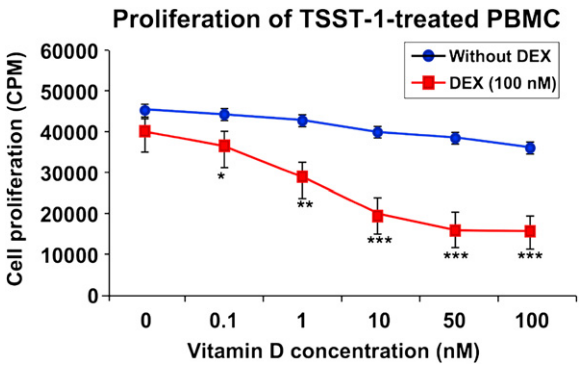

FIG 3. VitD augments DEX's effect on TSST-1–stimulated lymphocyte proliferation in human PBMCs. PBMCs from healthy control subjects were treated with DEX and VitD, as indicated in the Methods section. Tritiated thymidine was added to the medium 18 hours before collecting the cells. Cell division was estimated based on tritiated thymidine incorporation (n = 4).

The relationship found between aeroallergen sensitivity and lower VitD levels appears to be driven by perennial aeroallergens, house dust mite and dog in particular. Clinically, correlations between lower VitD levels and markers of allergy in childhood asthma have also been found by other investigators.[12] With respect to anaphylaxis, higher epinephrine autoinjector prescription rates have been shown in northern latitudes (a presumed area of low VitD levels) after controlling for socioeconomic factors.[23]

Importantly, our study also demonstrated significant associations between ICS use, oral corticosteroids use, and total steroid dose with lower VitD levels. Our data are consistent with those of a previous report of lower 25-hydroxyvitamin D levels in patients taking daily oral glucocorticoids at doses of 15 to 100 mg.[24] Cumulative glucocorticoid dose exposure has been associated with lower 25-hydoxyvitamin D levels in a more recent study on patients with systemic lupus erythematosus.[25] One explanation for our findings is that lower VitD levels contribute to increasing asthma severity and a concomitant need for escalating pharmacologic intervention, which frequently will entail inhaled and oral glucocorticoid administration. An additional novel possibility we considered is that VitD has effects on glucocorticoid pathways and VitD insufficiency promotes the need for higher doses of glucocorticoids to achieve treatment effect.

Upregulation of *MKP1* and *IL10* expression is essential for glucocorticoid-mediated anti-inflammatory and immunosuppressive effects. Xystrakis et al[26] reported that the addition of VitD and DEX to cultures of $CD4^+$ regulatory T cells from subjects with steroid-resistant asthma enhanced IL-10 secretion from these cells to levels comparable to those seen in cells of steroid-sensitive patients treated only with DEX. The study also demonstrated that VitD blocked DEX-induced downregulation of the glucocorticoid receptor in $CD4^+$ cells from healthy volunteers.[26] When 3 steroid-resistant subjects were given small doses of VitD for 7 days, all subjects showed enhanced *IL10* expression by $CD4^+$ cells.[26] This important finding has yet to be confirmed by other investigators.

In the current study, we tested whether VitD enhances glucocorticoid induction of *MKP1* and *IL10* in PBMCs using real-time PCR and T-cell proliferation assays. The data suggest VitD enhancement of glucocorticoid action in PBMCs in asthmatic patients *in vitro*. Similarly, significant enhancement in DEX-induced *MKP1* and *IL10* mRNA was observed after VitD pretreatment in PBMCs from healthy control subjects, suggesting that VitD's effect was not exclusive for asthmatic patients.[27] Also, *in vitro* data demonstrate that VitD addition can decrease the active dose of DEX greater than 10-fold. In an experimental model of steroid resistance induced by staphylococcal superantigen, the presence of VitD in culture restored the immunosuppressive function of DEX. The results of this study suggest that VitD supplementation might potentiate the anti-inflammatory function of corticosteroids in asthmatic patients.

Our study has several limitations. First, our clinical data were retrospective and relied on electronic medical record documentation. Given the emerging data on possible relationships between VitD and allergic diseases,[7,23,28] patients entering our day program during the study period routinely had VitD levels drawn, irrespective of provider concerns for rickets or dietary deficiencies. This minimized a bias toward lower VitD levels.

Second, the variability in the data, the sample size, and the nonnormal distribution of several variables made it difficult to create an efficient multivariate model to examine the strength of the correlations noted in our univariate analyses.

Third, our small sample size and the tertiary nature of our institution might limit the generalization of these findings to all US children with asthma. One advantage of our data set is that our patient's live in multiple locations. Even though our median latitude (39°N) was indicative of our institution's location, only 30% of our subjects resided in Colorado, and our data set included patients from a total of 28 different states representing a variety of northern latitudes (18°N-49°N).

In summary, our study provides important information on VitD levels and childhood asthma in higher northern latitudes. We were able to show that VitD insufficiency in childhood asthma is common and similar to that seen in the general population. We found significant correlations between several markers of atopy and lung function with VitD levels. Our study involved a high number of oral steroid−dependent asthmatic patients and demonstrated significant correlations between inhaled steroid use, oral steroid use, and total steroid dose with VitD levels. This finding supports our laboratory findings that VitD enhances the anti-inflammatory effects of glucocorticoids. These findings have important implications on potential future directions in asthma research. First, these findings should be confirmed in a prospective fashion that involves the generation of an efficient multivariate model. Second, further research should be directed at the use of VitD supplementation as a potential steroid-sparing agent in patients with moderate-to-severe persistent asthma, as well as a modifier of asthma disease severity.

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Clinical implications: Our study suggests that VitD supplementation might potentiate anti-inflammatory function of corticosteroids in asthmatic patients and thereby improve asthma control.

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